Genetic stability of micropropagated and cryopreserved silver birch

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Introduction

Silver birch (*Betula pendula* Roth) is an important raw material for plywood and pulp industry in Finland. Breeding of silver birch has started at the end of 1940's and silver birch was the first forest tree species micropropagated in commercial scale in Finland. The importance to preserve biodiversity within the species has been recognized. Conventional *in situ* and *ex situ* conservation methods of birch are already reality, and conservation of specific genotypes by *in vitro* methods, such as cryopreservation has been emphasised. Cryopreservation i.e. the storage of material at ultra low temperatures can be applied to avoid increased risks of contamination and somaclonal variation due to prolonged tissue culture. Before practical applications attention has to be paid on genetic fidelity testing and field evaluation of cryopreserved material. In this study we focus specific attention on the genetic stability of silver birch plants regenerated after tissue culture and after cryopreservation using different *in vivo* and *in vitro* protocols with slow cooling.

Material and methods

The study was performed on five birch clones, E1987, E5201, E5382, E5387, and E5398, with eight different types of material.

The material was collected from:

1) the over 60-year-old donor trees (Fig. 1A) and from plants regenerated after:

Fig. 1. A) Gathering twigs from a donor tree, B) *In vitro* shoot culture, C) Cryostorage.

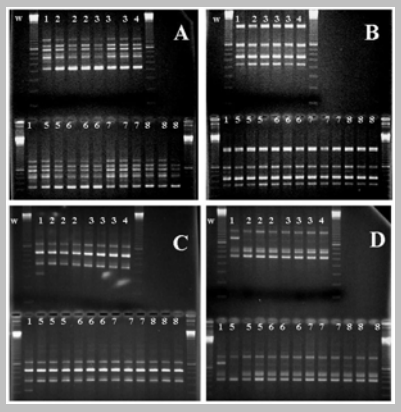

Fig. 2. Examples of the RAPD profiles generated for different birch clones. The samples marked with numbers 1-8 originate in the corresponding treatments, water (w) being used as negative control. A) Clone E1987 assayed with the OPE-19 primer, B) clone E5398 and the OPE-17 primer, C) clone E5387 and the OPF-03 primer, and D) E5387 with the OPF-15 primer. In most cases (A-B), no intraclonal variation was found. The most frequently observed aberration was the donor tree differing from the tissue-cultured and cryopreserved material of the same clone, as seen in C and D.

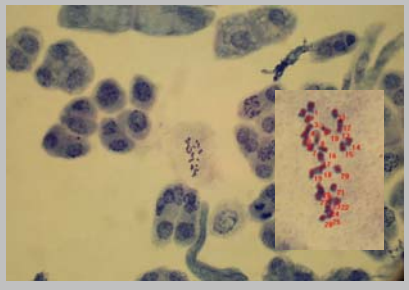

Fig. 3. Feulgen-Giemsa double-staining of chromosomes of young leaves.

2) micropropagation for five months (young cultures)
3) *in vivo* cryopreservation of winter buds from donor trees
4) micropropagation for five years (old cultures) (Fig. 1B)

and from plants regenerated after *in vitro* cryopreservation (Fig. 1C) of old cultures cold hardened with four different protocols;

5) at 4°C under SD either on standard WPM with BA or
6) under the same conditions on medium with BA substituted with ABA

Together with the same conditions and ABA substitution cold hardening was also performed on WPM containing either

7) 10 mM KNO_3 or
8) 7 mM L-glutamine
as an only nitrogen source.

Genetic stability of birch material was tested in three different levels: *1*) the RAPD markers were utilised to detect mutations and genomic rearrangements, *2*) chromosome analysis was performed to examine the whole genome, and 3) growth and morphology of the regenerated plants was followed in a field test during several growth seasons.

Results and discussion

According to the results obtained so far, there seems to be no systematic effect of any of the tested treatments on the genetic stability of silver birch. In RAPD assays, some intraclonal variation was found. The observed differences concentrated on certain clones, and most often it was the donor-tree that showed a differing profile compared with the tissue cultured and cryopreserved material of the same clone (Fig. 2). This might reflect somaclonal variation existing in mature trees. Confirmation of the reproducibility of the observed RAPD variations is currently under the progress, as are also the chromosome analysis. Genome size of birch is relatively small (470Mb), 2n = 28. Chromosome counting has not revealed polyploidy (Fig. 3) although multiplication takes place via adventitious buds differentiating from the basal callus of stems. Tissue culture age or cryopreservation protocol used did not seem to affect the growth of regenerated plants. Plants in all treatments show normal morphology (Fig. 4).

Fig. 4. Regenerated plants in summer 2001.